※ (22)

Written Amendment

(Amendment under Article 11 of Patent Law)

To: NANAJO Satomi,

Examiner of the Patent Office

1. Designation of the International Application

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2. Applicant

Name: YANAGA Hiroko

Address: Ambient Kokura 912, 16-1, Kumamoto 3-chome Kokurakita-ku, Kitakyusyu-shi, Fukuoka 802-0044 JAPAN

Nationality: Japan

Residence: Japan

3. Agent

Name: NARUSE katsuo

Patent Attorney (8273)

Address: 5th Floor, TKK Nishishinbashi Bldg., 11-5, Nishishinbashi 2-chome, Minato-ku, Tokyo 105-0003 Japan

4. Subject to be amended: Description

5. Contents of Amendment

(1) The statement "The thus obtained tissue is fed into an injection syringe or the like and, after attaching a needle, injected into a defect in a cartilage to thereby treat or repair nasal deformation, nose elevation,

facial bone deformation, facial bone defect, gnathoplasty, skull deformation, skull defect, arthrosis deformans, microtia and other diseases accompanied by a defect in a cartilage and a defective cartilage." (description, page 6, lines 6 to 9), is amended as follows by deleting the term "arthrosis deformans", "The thus obtained tissue is fed into an injection syringe or the like and, after attaching a needle, injected into a defect in a cartilage to thereby treat or repair nasal deformation, nose elevation, facial bone deformation, facial bone defect, gnathoplasty, skull deformation, skull defect, microtia and other diseases accompanied by a defect in a cartilage and a defective cartilage."

(2) The statement "The method of producing chondrocytes according to the present invention can be applied to the culture of chondrocytes of any human cartilage tissue having perichondrium bonded thereto such as auricular cartilage, costal cartilage, articular cartilage, intervertebral cartilage or tracheal cartilage. In particular, it is suitable for culturing and proliferating chondrocytes of auricular cartilage." (description, page 6, lines 19 to 22) is amended as follows by deleting the term "articular cartilage", "The method of producing chondrocytes

according to the present invention can be applied to the culture of chondrocytes of any human cartilage tissue having perichondrium bonded thereto such as auricular cartilage, costal cartilage, intervertebral cartilage or tracheal cartilage. In particular, it is suitable for culturing and proliferating chondrocytes of auricular cartilage."

(3) The statement "The thus treated tissue is centrifuged and the obtained precipitate is employed in the culture." (description, page 7, line 10) is amended as "The thus treated tissue is centrifuged and the obtained precipitate (chondrocytes and perichondrium cells) is employed in the culture.

6. List of Attached Documents

(1) Description page 6

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it is possible to obtain a tissue which has a high mechanical strength and therefore is durable handling with instruments such as tweezers and never undergoes dispersion or absorption *in vivo* after transplantation. Although the number of multilayer seedings varies depending on the size of a desired tissue, it is generally preferable to carry out the multilayer seeding three or four times.

The thus obtained tissue is fed into an injection syringe or the like and, after attaching a needle, injected into a defect in a cartilage to thereby treat or repair nasal deformation, nose elevation, facial bone deformation, facial bone defect, gnathoplasty, skull deformation, skull defect, microtia and other diseases accompanied by a defect in a cartilage and a defective cartilage. In this step, the cartilage tissue may be used in the form of a mixture with a carrier selected from among collagen, polyglycolic acid (PGA), polylactic acid, an alginate, polyethylene oxide, a fibrin adhesive, a polylactic acid-polyglycolic acid copolymer, a proteoglycan and a glucosaminoglycan. The chondrocytes obtained by the production method according to the present invention are practically usable as such without resort to a carrier.

A. Human chondrocytes

The method of producing chondrocytes according to the present invention can be applied to the culture of chondrocytes of any human cartilage tissue having perichondrium bonded thereto such as auricular cartilage, costal cartilage, intervertebral cartilage or tracheal cartilage. In particular, it is suitable for culturing and proliferating chondrocytes of auricular cartilage.

The chondrocytes to be used in the production method according to the present invention can be obtained from a human cartilage tissue having perichondrium by publicly known methods. It is generally

preferable that an excised cartilage tissue is diced with a surgical knife or the like, treated with collagenase and then cultured and proliferated. For example, the process can be performed as follows.

1) A cartilage tissue is excised and disinfected by allowing to stand at about 4°C overnight together with an antibiotic (for example, penicillin or kanamycin) or an antifungal agent (for example, amphotericin B). Then, the cartilage tissue is diced with a surgical knife, etc.

2) The diced cartilage tissue is transferred into a medium containing type II collagenase and allowed to stand at about 4°C overnight. Next, it is shaken at 37°C for 4 hours.

3) The thus treated tissue is centrifuged and the obtained precipitate (chondrocytes·perichondrium cells) is employed in the culture.

By this method, 3 to 5×10^6 chondrocytes can be obtained at the first generation of subculture from a human auricular cartilage tissue piece (1 cm^2). In the culture method according to the present invention, moreover, use can be made of a known growth factor, especially one capable of stimulating the proliferation of cartilage, appropriately selected form among FGF (for example, bFGF), IGF (for example, IGF-I), bone morphogenetic protein 9 (BMP9) and combinations thereof.

B. Method of culturing human chondrocytes

To culture human chondrocytes, use can be made of publicly known media suitable for culturing chondrocytes. In addition to fetal bovine serum (FBS) or human serum and hydrocortisone, the media may optionally contain a proliferation factor such as human bFGF or human IGF-I (Cuevas et. al., Biochem. Biophys. Res. Commun. 156, 611-618 (1988);and Froger-Gaillard et al., Endocrinol. 124, 2365-72). As an example of such a medium, DME(H) medium containing FBS (preferably about 10%), human bFGF (preferably